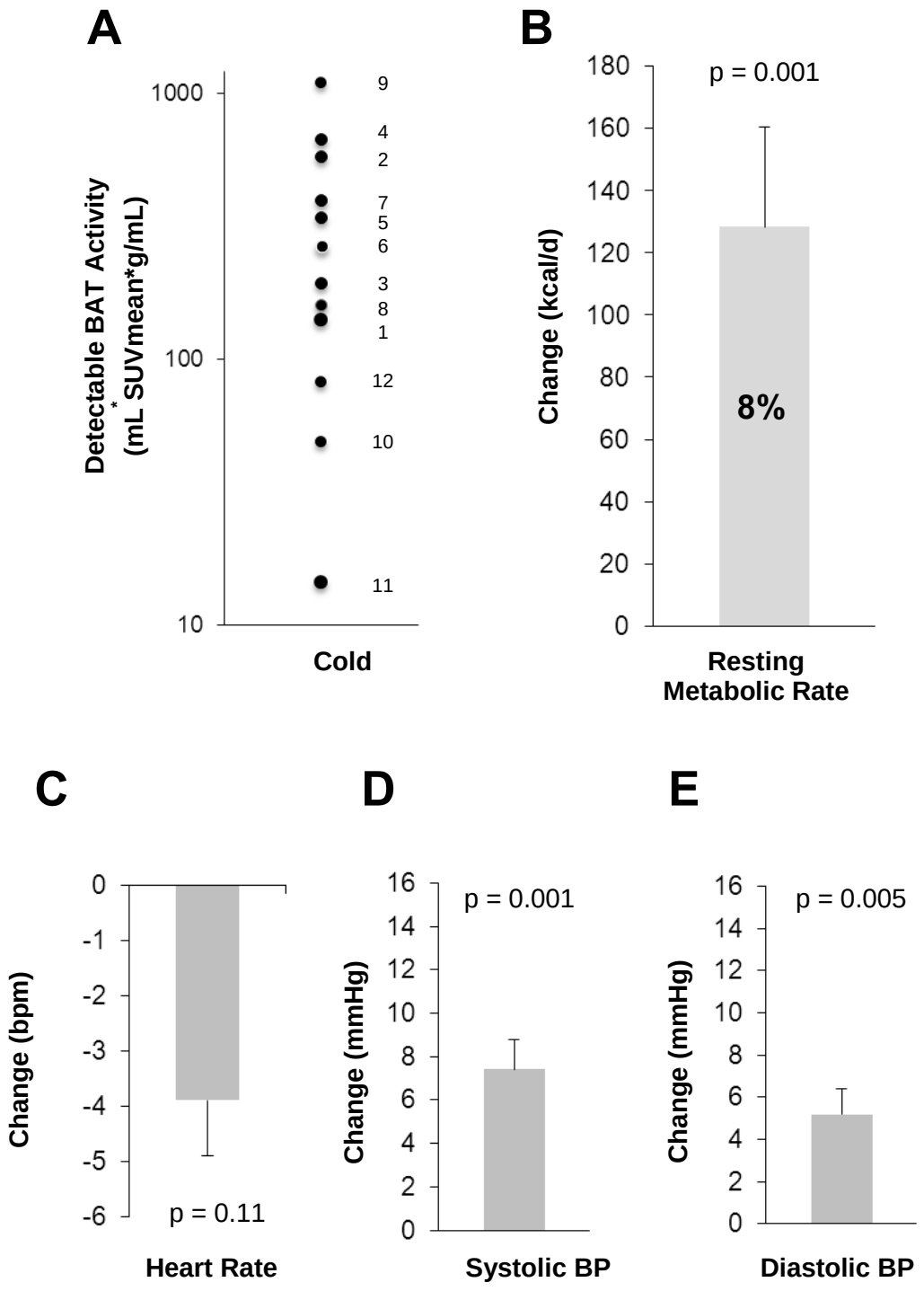

Figure S1, Related to Table 1. **Cold-Induced Changes in BAT Activity, Resting Metabolic Rate, and Vital Signs.**

(A) ^{18}F-FDG uptake seen via PET/CT; numbers correspond to subject identification number in Figure S2.

(B) Resting metabolic rate (RMR).

(C) Heart rate.

(D) Systolic blood pressure (BP).

(E) Diastolic BP.

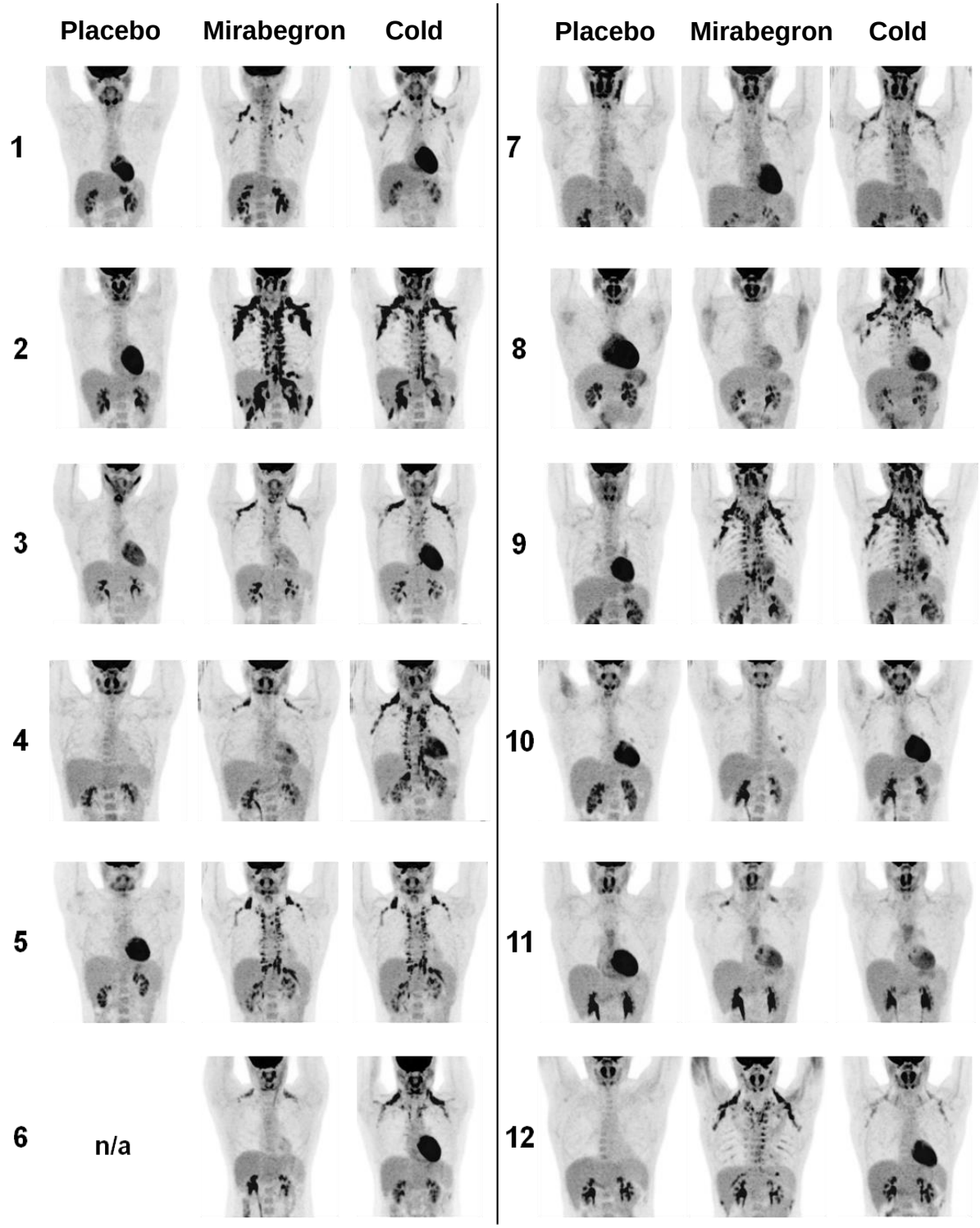

Figure S2, Related to Figure 1. Tissue Glucose Uptake in Response to Placebo, Mirabegron, or Cold. Shown are the differences in total-body 18F-FDG uptake via PET in all twelve subjects when they were given placebo (left), 200 mg of the β3-adrenergic receptor agonist mirabegron (center), or mild cold (right). Of note, subject #6 withdrew from the study several weeks after the efficacy of mild cold exposure and mirabegron were evaluated and did not receive placebo.

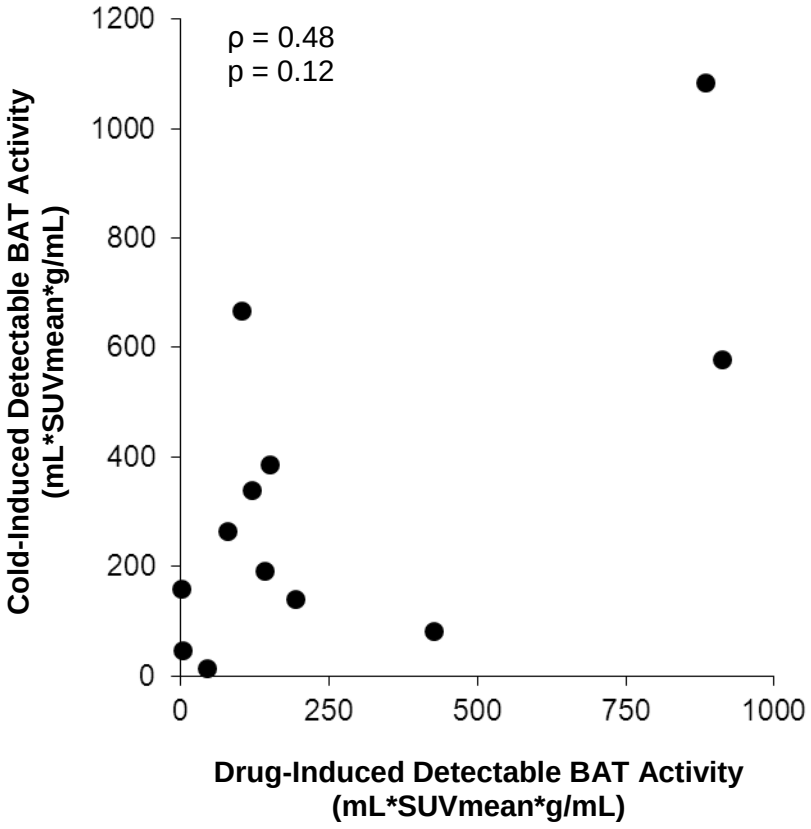

Figure S3, Related to Figure 3. Correlation Between Cold- and Drug-Induced BAT Metabolic Activity. Shown for the twelve subjects is the relationship between cold- and 200 mg mirabegron-induced detectable BAT activity.

.

Table S1, Related to Figure 2. Metabolite Concentrations[A]

Metabolite (units)	Placebo	Mirabegron	P value[B]
Glucose (mg/dL)	79 (76-86)	87 (82-90)	0.08
NEFA (mEq/L)	0.40 (0.35-0.55)	0.70 (0.60-0.83)	0.05
Lactic Acid (mg/dL)	6.4 (5.6-7.7)	5.8 (5.2-7.4)	0.34
β-hydroxybutyrate (mM)	0.074 (0.068-0.193)	0.329 (0.160-0.563)	0.16
Insulin (μU/mL)	5.06 (3.56-6.19)	7.61 (6.90-8.66)	0.004
C-peptide (ng/mL)	1.8 (1.5-2.0)	2.2 (1.8-2.3)	0.004
HOMA-IR (mM*μU/mL)[C]	0.99 (0.68-1.35)	1.66 (1.35-1.77)	**0.002**
Glucagon (pg/mL)	46 (45-67)	55 (45-68)	0.27
Norepinephrine (pg/mL)	35 (13-143)	73 (13-122)	0.22
Cortisol (μg/dL)	7.8 (6.3-9.3)	7.8 (6.4-9.1)	0.87
TSH (μIU/mL)	1.33 (0.91-1.75)	1.27 (0.90-1.73)	0.48
Free T4 (ng/dL)	1.28 (1.19-1.39)	1.25 (1.16-1.38)	0.98
Total T3 (ng/dL)	103 (94-110)	106 (90-115)	0.78

[A]Values are median (interquartile range)

[B]Values in bold indicate *P* values ≤ 0.002, the threshold for a Bonferroni correction with 21 comparisons.

[C]Calculated as ((insulin concentration (μU/mL) * glucose concentration (mM))/22.5)

Supplemental Experimental Procedures

Study Population

The following were the inclusion criteria: healthy men between ages 18 and 65 years with BMI between 18 and 40 kg/m^2. We focused our recruitment on younger, leaner men since they were more likely to have detectable cold-stimulated BAT. Volunteers could not have participated in a clinical trial and received either an investigational or a marketed drug, or donated blood, within two months prior to the start of the study. The exclusion criteria were the following: women; history of any local or systemic infectious disease with fever or requiring antibiotic within four weeks of drug administration; a QTc interval above normal or the current use of any concomitant QT-prolonging drug; a clinically-significant abnormal ECG; a laboratory test result that is outside more than 1.5-fold outside the normal range and/or was judged to be clinically significant; current addiction to alcohol or substances of abuse; mental incapacity, unwillingness or language barriers precluding adequate understanding or cooperation; the use of a course of systemic corticosteroids or other medication known to cause insulin resistance in previous six weeks; the use of any medication known to be a CYP2D6 substrate; a diagnosis of bladder outlet obstruction or the use of antimuscarinic medications for the treatment of overactive bladder; and the use of beta adrenergic receptor blockers or calcium channel blockers. Since BAT activity is affected by the sympathetic nervous system and may be modulated by thyroid function, the following conditions were contraindicated: subjects with hyperthyroidism or hypothyroidism, hypertension (even if controlled with medications), heart disease (including CAD and CHF), cardiac arrhythmias, diabetes, unstable vasomotor system, or those taking monoamine oxidase inhibitors. Because the goal was to study adult human brown adipose tissue, children were not eligible to participate. Special classes of subjects, such as prisoners, institutionalized individuals, or others who may be considered vulnerable populations were also not involved in this study.

Of note, one subject withdrew from the study several weeks after the efficacy of mild cold exposure and mirabegron were evaluated and did not receive placebo.

Clinical Measurements

We measured blood pressure and heart rate using a SureSigns VS3 vital signs monitor (Philips Healthcare). We assessed calculated metabolic rate (RMR) using a Sensormedics Vmax Encore 29 (VIASYS Respiratory Care, Inc.). We measured body composition via dual-energy x-ray absorptiometry (DXA) whole-body scanner (Discovery A (SIN 86195), Hologic, Inc). Plasma glucose was measured in the BIDMC clinical laboratory. Serum levels of insulin, C-peptide, cortisol, and TSH were measured at the Massachusetts General Hospital Clinical Laboratory Research Core (http://catalyst.harvard.edu/services/hccrc-lab/). Plasma norepinephrine concentrations were measured via HPLC at the Mayo Clinic (Mayo Medical Laboratories); for the four subjects whose concentrations were below the assay detection limit, we imputed the half-minimum value. Serum non-esterified fatty acids (NEFA), lactic acid, free T4, total T3, and glucagon were measured using standard procedures at the Laboratory Corporation of American (Burlington, NC). Serum β-hydroxybutyrate was measured using the LiquiColor kit (Stanbio Laboratories) by the JDC Specialized Assay Core.

Study Day and Imaging Protocol

We asked subjects to continue their standard weight-maintenance diets over the course of their participation and to refrain from caffeine and alcohol intake for 48 hours prior to each study day. The evening before a study day, subjects were admitted to the BIDMC clinical research center (CRC) and began fasting from 12 am onward. Room temperature was maintained above 23 °C. Upon waking the next morning, subjects donned a standard hospital scrub suit. We recorded vital signs every 15 minutes until the end of the study day. RMR was measured 30 minutes after the subjects awoke, and depending on the study day, we gave one of two stimuli:

a single oral dose of 200 mg mirabegron or placebo. After 210 minutes, we drew blood to measure metabolite levels, and then administered an intravenous bolus of 444 MBq (12 mCi) of [^{18}F]-2-fluoro-D-2-deoxy-D-glucose (FDG). We chose this timing because it represented the peak plasma concentration of mirabegron based on previous clinical trials (Malik et al., 2012). We then measured RMR a second time. For each subject, we compared the average systolic blood pressure (BP), diastolic BP, and heart rate (HR) over two time intervals: during the hour prior to intervention (3 measures) and then for placebo and mirabegron, from 210 to 250 minutes afterward (3 measures), which represented the time of predicted peak activity of mirabegron (Malik et al., 2012). Of note, for cold exposure, the second time interval was from 60 to 90 minutes (3 measures) after the initiation of cooling, which corresponds to the period when the radiotracer was distributing through the body (Cypess et al., 2012).

Sixty minutes after ^{18}F-FDG injection, images were acquired using a Discovery LS multidetector helical PET–CT scanner (GE Medical Systems) as described (Cypess et al., 2012). We classified tissue as BAT in each axial slice on a pixel-by-pixel basis when CT was in the range of -250 to -10 Hounsfield Units and when the Standard Uptake Value (activity in MBq per unit pixel volume of 64 μL (Cypess et al., 2009) within the region of interest divided by the injected dose in MBq per body mass in g) was ≥ 1.5 (SUV*g/mL). Although a ratio of two activities, the denominators are different, so the SUV therefore has the units of "SUV*g/mL": [SUV *(~~mCi~~/mL)/(~~mCi~~/g) → SUV*g/mL]. BAT activity in each pixel was defined as the product of the volume of BAT in mL and its mean SUV (mL*SUVmean*g/mL).

We calculated a subject's total detectable BAT metabolic activity by taking the sum of the measured BAT activity in the principal cervical, supraclavicular, and anterior thoracic adipose tissue depots from vertebral level C3 to T7 as well as the BAT in the paraspinal, periaortic, perihepatic, perirenal and perisplenic fat depots. We calculated the change in drug- and cold-induced BAT activity by taking the difference between the activities measured during the

placebo- and drug-dosing or cold-exposure days. Maximal glucose uptake (SUVmax) in the myocardium, subcutaneous abdominal WAT, erector spinae skeletal muscle, and liver were quantified as described (Cypess et al., 2012). In brief, we identified the SUVmax measured in regions of interest in six consecutive axial slices in the left ventricle, white adipose tissue of the subcutaneous lumbar region, erector spinae skeletal muscle, and right lobe of the liver.

LC-MS/MS Analyses of Mirabegron

Detection and quantitation of mirabegron was achieved by LC-MS/MS analysis performed with an Agilent (Agilent Technologies) 6460 triple-quad LC/MS system using an atmospheric pressure chemical ionization (APCI) source in the positive ion mode. An isocratic HPLC method was used using a Phenomenex Kinetex 2.6 µm, C18, 50 x 2.1 mm column at a flow rate of 0.2 mL/min with solvent (A): 20 mM ammonium acetate and solvent (B): acetonitrile run as an isocratic mixture at 30% B for 3 minutes. A multiple reaction monitoring (MRM) method was used to monitor the MS/MS transitions of the $[M+H]^+$ ions for mirabegron and the internal standard [$^{13}C_6$] mirabegron to their corresponding most abundant fragment ions (397.2->379.1 m/z) and [403.2->385.1 m/z], respectively. Mass spectrometer parameter settings were as follows: counter-current gas temp (350 °C) and flow rate 5 L/min, nebulizer pressure (60 psi), APCI heater (300°C), and capillary (4500V). A standard curve was generated using serial dilutions of mirabegron between 0.2-1000 pg/µL with a fixed amount of [$^{13}C_6$] mirabegron of 100 pg/µL.

Sample Size Calculation and Statistical Analysis

In our prior studies of cold-exposed men, $\log_{10}$ BAT activity following cold exposure was normally distributed with a mean of 1.47 and standard deviation of 0.72. By studying ten subjects, we estimated to have 80% power (α=0.05) to detect a difference in the mean response of matched pairs to mirabegron vs. placebo equal to -.716 or +.716, which corresponded to 50%

in the log scale and 20% in the natural scale of the efficacy of cold activation. In our previous studies, the rate of detection of cold-activated BAT was 66%, so we estimated that studying fifteen subjects with cold exposure would yield at least ten subjects with detectable BAT activity who could proceed to evaluation of placebo and 200 mg mirabegron. Of note, the difference between placebo and drug-induced $\log_{10}$ BAT activity, the endpoint used in the power calculation, had a $p < 0.001$ when using Student's *t*-test. The difference in the non-transformed BAT activity had a $p = 0.001$ when using Wilcoxon sign-ranks test.